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Case Report

Demonstration of McCune-Albright mutations in the liver of children with high γGT progressive cholestasis

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Two patients presented with neonatal cholestasis and acholic stools as first manifestations of McCune-Albright syndrome. Both went through an extensive evaluation including an exploratory laparotomy with peroperative cholangiography which ruled out biliary atresia. One patient presented from the fourth month of life with the classical café-au-lait spots following Blaschko's lines, while less classical café-au-lait spots were seen in the second patient at the age of 4 years. Bone lesions were seen in one patient at the age of 2.5 years and in the other at the age of 4 years. Despite the severity of presentation, both patients cleared their jaundice within 6 months, but still had mild abnormalities of liver function tests. Both patients showed an activating mutation of codon 201 in the gene encoding the α-subunit of the G-protein that stimulates adenylcyclase in liver tissue, suggesting that this metabolic defect could be responsible for the cholestatic syndrome. Similar mutations have been found in other affected tissues in patients with the McCune-Albright syndrome. We propose that McCune-Albright syndrome be included in the list for differential diagnosis of neonatal cholestasis and chronic cholestasis of infancy, as a rare cause.

Key words: **Café-au-lait spots; McCune-Albright syndrome; Mutation of arginine 201 of the α-Gs protein; Neonatal cholestasis; Polyostotic fibrous dysplasia.**

The McCune-Albright syndrome (MAS) is characterised by a clinical triad of signs: café-au-lait spots, polyostotic fibrous dysplasia and sexual precocity, but the first two and/or a hyperfunctional endocrinopathy are enough to make the diagnosis (1,2).

The severity of the disease is variable. The syndrome has generally been considered non-fatal, and whilst some long-term follow-up studies suggested that patients were at no increased risk for nonendocrine disease or premature death (3), others, however, indicated an increased risk (4). Hepatobiliary dysfunction is included in the nonendocrine abnormalities and although it appears to be a rare manifestation, severe neonatal jaundice, persistent elevated serum liver enzymes and liver cirrhosis have been described, alone or in combination (4).

In patients with the MAS an activating mutation has been identified in the gene for the alfa subunit of Gs, the G protein that stimulates cyclic adenosine monophosphate formation (5–8). This mutation has been variably documented in different affected endocrine and nonendocrine tissues, including liver specimens, consistent with the mosaic distribution of abnormal cells generated by somatic cell mutation early in embryogenesis (4,5). The occurrence and severity of bone, skin, endocrine and nonendocrine abnormalities in a specific patient would depend on the number and location of cells bearing the mutation (5). The mechanism by which activated Gs might cause hepatobiliary dysfunction remains unknown.

In recent years molecular biology has allowed differentiation of previously phenotypically similar types of progressive familial intrahepatic cholestasis (PFIC). Mutations of three genes are now recognized in these patients (PFIC-1, PFIC-2/SPGP, MDR3).

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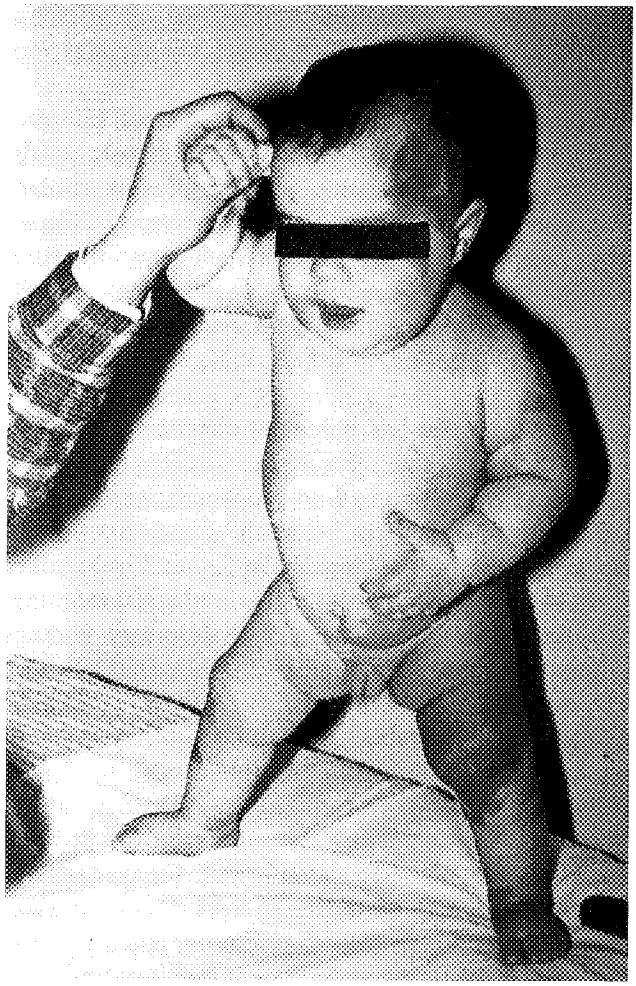

Fig. 1. Case 1 – Café-au-lait spots following Blaschko's lines on the face, thoracoabdominal region, arms and legs.

The gene for Byler disease (PFIC-1) has been mapped to a 19-cM region of 18q21–q22 (9). Children who have a clinically similar disorder, but who are not members of the Amish kindred in which Byler disease was described, are said to have Byler syndrome. A different locus designated PFIC-2 has been mapped on chromosome 2q24 in patients with Byler syndrome of unrelated non-Amish families (10). The PFIC2 gene has been identified by mutations in a positional candidate, human bile salt export pump, which encodes a liver-specific ATP-binding cassette (ABC) transporter, sister of p-glycoprotein (SPGP) (11). The phenotype of PFIC-1 and PFIC-2 linked group is consistent with defective bile acid transport at the hepatocyte canalicular membrane. Mutations in the MDR3 gene were found in patients with another form of progressive familial intrahepatic cholestasis with high γGT (12).

We report two patients with neonatal cholestasis of the high γGT subtype for which the McCune-Albright mutations (R 201 C or R 201 H) were identified in liver tissue. This is a rare cause but may provide new insights into the mechanism of neonatal cholestasis.

Case Reports

Case 1

A male Portuguese infant was born to non-consanguineous parents after a normal 39-week pregnancy. Birth weight was 2900 g, length 48 cm and head circumference 34 cm. Jaundice was noted from the 2nd day of life. He was discharged home on the 4th day, after 48 h phototherapy and readmitted on the 8th day for massive umbilical bleeding. He was pale and jaundiced. The liver edge was palpable 3 cm below the costal margin and the spleen was not enlarged. There were no signs of umbilical infection. Cardiovascular and pulmonary auscultation were normal. The fundi were normal and he showed no peculiar facies. We observed acholic stools and dark urine. Laboratory findings on admission confirmed cholestasis: total bilirubin 185 μmol/l; conjugated bilirubin 180 μmol/l; AST 380 UI/l (n<56); ALT 427 UI/l (n<39); γGT 1240 UI/l (n<45);

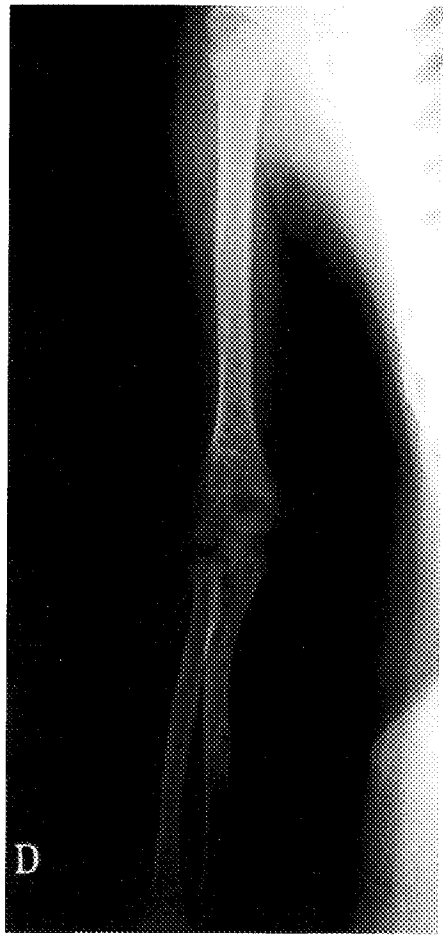

Fig. 2. Case 1 – Lesions of polyostotic fibrous dysplasia in the right arm.

total serum proteins, serum albumin and total cholesterol were normal; blood and urine cultures were negative; Hb 10.6 g/dl (mean 16.5 g/dl; −2SD 12.5 g/dl); WBC 9560/mm^3 (13000±6000), platelets 225000/mm^3 (150000–400000); prothrombin time 17 s (N 14 s). Coagulation tests normalised after vitamin K administration. Further investigations ruled out the most common causes of cholestasis (13). Serum and urinary bile acids were not performed. The HIDA scanning showed absence of intestinal contrast despite optimal parenchymal captation. An exploratory laparotomy with peroperative cholangiography was performed at 8 weeks of age. The extrahepatic biliary tree was patent and normal. Liver biopsy showed giant cell transformation with mild portal inflammatory infiltrate and normal bile ducts. The infant was discharged home with the diagnosis of idiopathic neonatal hepatitis.

Jaundice subsided spontaneously by 6 months. From the 4th month of life he presented well-defined café-au-lait spots following Blaschko's lines on the face, thoracoabdominal and dorsal regions, arms and legs predominantly on to the right side of the body (Fig. 1). The diagnosis of McCune-Albright syndrome was suspected but skeletal survey was normal. By 2.5 years of age a complete X-ray film showed lesions of polyostotic fibrous dysplasia most marked in the arms (Fig. 2) and the diagnosis of McCune-Albright syndrome was then made. The child is now 11 years old and has neither bone fractures nor endocrinological dysfunction. He has no pruritus. Liver function tests remain abnormal: AST 83 UI/l; ALT 122 UI/l; γGT 202 UI/l. Liver biopsy shows near normal hepatic parenchyma. Recently we have been able to identify in this patient, an Arg 201-to-Cys mutation in the gene for the alpha subunit of Gs protein in liver tissue (Fig. 3).

Case 2

A female Greek infant was born to non-consanguineous parents after a normal 42-week pregnancy. Birth weight, length and head circumference were on the 50th percentile. Because of conjugated hyperbilirubinemia from the 4th day of life (total bilirubin 212.5 μmol/l, conjugated bilirubin 136 μmol/l) and unexplained raised serum liver enzymes she underwent a liver biopsy at 4 weeks of age, which showed a typical image of neonatal giant cell hepatitis.

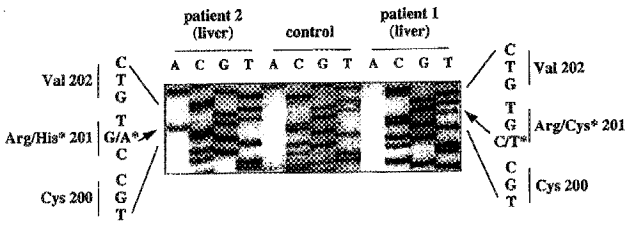

Fig. 3. Mutations identified in both patients.

At 3 months she remained jaundiced (total bilirubin 93.5 μmol/l, conjugated 81.6 μmol/) with pale stools. An exploratory laparotomy with peroperative cholangiography showed a permeable extrahepatic biliary tree. Liver biopsy showed giant cell transformation with some Councilman bodies and inflammatory infiltrates in the hepatic parenchyma, and the portal areas exhibited a mild inflammatory infiltrate and normal bile ducts.

At 6 months she had persistent abnormal liver function tests, despite complete resolution of jaundice. Pruritus was absent. She had no peculiar facies. The liver edge was palpable 4 cm below the costal margin and the spleen was not enlarged. Cardiac and pulmonary auscultation were normal. Ophthalmological examination detected no abnormalities. Laboratory findings showed: total bilirubin 12 μmol/l; conjugated bilirubin 7.5 μmol/l; AST 132 UI/l; ALT 197 UI/l; γGT 1434 UI/l; total serum proteins, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were normal; Hb −9.2 g/dl (mean 12.0; −2SD 10.5); WBC 11090/mm^3 with normal formula; platelets 463000/mm^3; coagulation tests were normal. Further investigations ruled out the most common causes of cholestasis (13) and included normal serum bile acids analysed by fast atom bombardment and tandem mass spectrometry (14). The infant was discharged on ursodeoxycholic acid therapy with the diagnosis of idiopathic neonatal hepatitis. At follow-up she revealed normal growth and absence of jaundice or pruritus. Abnormal liver enzymes persisted. Compliance to ursodeoxycholic acid therapy was irregular. Liver biopsy performed at 13 months and 2.5 years of age showed discrete portal fibrosis and inflammatory infiltrates, with normal bile ducts. Viral cultures of liver specimens were negative.

At 4 years of age a fracture of the right femur neck was diagnosed and two large café-au-lait spots were noted in the sacrum region. A complete skeletal survey showed polyostotic fibrous dysplasia with most lesions on the right-sided bones. The diagnosis of McCune-Albright syndrome was then made. Because of the high γGT an endoscopic retrograde cholangiopancreatography was performed and excluded sclerosing cholangitis. She is currently 9 years old and clinical progress has been marked by recurrent fractures of the right femur neck but absent endocrinological dysfunction. Slight abnormalities of liver function tests persist (AST 54 UI/l; ALT 102 UI/l; γGT 105 UI/l), in spite of a near normal liver histology. Recently we were able to identify in this pa-

tient, an Arg 201-to-His mutation in the gene for the alpha subunit of Gs protein in liver tissue (Fig. 3).

Method assay

DNA extraction: For both patients, DNAs were obtained from paraffin-embedded liver biopsies. The method is adapted from a previously described protocol (15). Briefly, 10-μm-wide sections of fixed embedded tissues are extracted twice with xylene to remove the paraffin. Samples are then washed twice with 100% ethanol, dried under vacuum and submitted to proteinase K digestion, for 2 h at 55°C, in 100 μl of digestion buffer (50 mM Tris pH 8.5, 1 mM EDTA, 0.5% Tween 20) containing 200 μg/ml of proteinase K. Proteinase K was then inactivated for 9 min at 95°C.

Amplification of DNA by polymerase chain reaction (PCR): Proteinase K-treated samples were centrifuged for 5 min at 13000 *g*. One to five microlitres of supernatant were used for PCR. Reactions were performed in a final volume of 50 μl containing 50 ng of each primer.

Exon 8 of the Gs alpha gene (GNAS1) (which contains codon 201) was amplified using the following pair of primers: Sense primer – 5′ GTG ATC AAG CAG GCT GAC TAT GTG 3′ and antisense primer – 5′ TAA CAG TTG GCT TAC TGG AA 3′), 200 μM of each nucleotide (Promega, France), 2.5 mM $MgCl_2$, 10 mM Tris (pH 8.3), 50 mM KCl, 0.01% gelatin and 0.2 U of Taq Polymerase (Promega, France). Samples were cycled for 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C for 35 cycles. Correct sizes of PCR products were verified by electrophoresis on agarose gel.

Sequencing of PCR products: Direct sequencing of one strand was performed with the sense primer using the Sequenase PCR product kit (Amersham Life Science, Cleveland, USA) according to the manufacturer's instructions. Reactions were repeated at least twice on different PCR products to avoid contamination artefacts. The products of sequencing reaction were migrated in 6% denaturant polyacrylamide gel and exposed to autoradiography. Negative and normal controls were included in every assay. In patient 1, an Arg to Cys (position 201) substitution was identified while in patient 2, the other substitution reported in McCune-Albright syndrome (Arg to His mutation) was documented.

Discussion

Progressive familial intrahepatic cholestasis is a heterogeneous group of inherited disorders with severe cholestatic liver disease from early infancy, for which mutations of three genes have been identified (9–12).

The McCune-Albright mutations identified in the liver tissue of our patients have so far not been considered in the general discussion over "genetic cholestasis". This mutation leads to an abnormal alpha subunit of the G protein that stimulates cyclic adenosine monophosphate formation. This molecular defect may play a role in the pathogenesis of cholestasis, possibly by interfering with normal biliary excretion by the liver cell (4). The extra- and intrahepatic bile ducts were found patent in both patients, confirming that the defect lies at the level of hepatocyte and/or biliary canalicular membrane, with possible interference by the abnormal protein with the secretion of normal biliary components. Abnormal embryological development of the biliary tract is excluded by the normal cholangiography in our patients.

Our description is in agreement with the severe cholestasis reported in the original descriptions of McCune (1) and Albright (2) in 1937. Nonendocrine abnormalities, including hepatobiliary dysfunction, have since been regularly reported (4).

An activating mutation in the gene encoding the alpha subunit of Gs protein has been variably documented in different affected endocrine and nonendocrine tissues, including liver specimens of two patients with neonatal cholestasis (4,5).

This pattern is consistent with the mosaic distribution of abnormal cells generated by a somatic cell mutation early in embryogenesis. The occurrence and severity of bone, skin, endocrine and nonendocrine abnormalities in a given patient would depend upon the number and location of cells with the mutation (4–8).

The McCune-Albright mutations identified in the liver tissue of our patients were an Arg to Cys (position 201) substitution in patient 1, while the other substitution reported in McCune-Albright syndrome (Arg to His mutation) was found in patient 2. The sequential patterns observed point to a mosaicism, a feature always present in MAS, given the post-zygotic origin of the mutation. For this reason DNA analysis of the parents is not required. In fact, to our knowledge, cases are always sporadic with no familial incidence ever reported. Hereditary transmission and thus constitutional mutation would most probably be lethal. Study of DNA from lymphocytes was not considered because the identification of the mutation in the liver is sufficient for the diagnosis. In addition, the mutation is rarely found in the lymphocytes and a negative result therefore would not exclude the diagnosis.

In patients with neonatal cholestasis, identification of the mutation in the liver may be the only initial diagnostic marker since bone lesions occur later and skin spots following Blaschko's lines (16) are not always as characteristic as in case 1.

The outcome of liver disease in previously described patients is not known (3,4). One patient underwent a liver transplant for end-stage liver disease attributed to hepatitis C (17). The course of the hepatic involvement in our patients has so far been benign despite the severity at presentation. Both patients, although they have mild abnormalities of liver function tests, have normal bilirubin with neither progressive fibrosis nor liver failure.

One of our patients was treated with ursodeoxycholic acid, which may have influenced the natural course of the disease (18).

We conclude that the McCune-Albright mutations, leading to an intrahepatic activation of an abnormal protein, are additional genetic and molecular defects causing cholestasis in infancy.

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